Section - IV

ORAL DRUG DELIVERY

1. Introduction

Prolonged administration of NSAIDs in chronic diseases such as arthritis exhibits several undesired side effects; like GIT irritation and ulceration which still represent an unsolved therapeutic problem. The development of a GIT-friendly anti-inflammatory therapy for the treatment of disease of joints presents a unique challenge. Considerable attention has been focused on the development of bioreversible derivatives, such as prodrugs, to temporarily mask the acidic group of NSAIDs as a promising means of reducing or abolishing the GI toxicity due to the local action mechanism.

Different approaches used for prodrug designing of NSAIDs are as follows:

1. Ester and amide prodrugs

Most prodrugs of NSAIDs have been prepared by derivatization of the carboxyl group. The esters have dominated prodrug research because they have the ideal characteristic of exhibiting reasonable *in vitro* chemical stability which allows them to be formulated with adequate shelf-lives. Additionally, by virtue of their ability to function as substrates for esterases, esters are suitably labile, *in vivo*[1]. With this aim different pro-moieties have been taken into consideration to design new efficacious NSAID prodrugs.

2. Anhydride prodrugs of NSAIDs

Unlike the ester bond used in prodrugs, anhydride bond is more susceptible to hydrolysis and is less sensitive to enzymatic action than the ester and amides.

3. A mutual prodrugs of NSAIDs

A mutual prodrug is a kind of prodrug in which the carrier used is another biologically active drug instead of some inert molecule. Some of the mutual prodrug examples are as follow:

3.1. Benorylate: This was the first mutual prodrug of NSAID in which coupling of paracetamol with aspirin minimized ulceration.

3.2. Coupling with amino acids: It is a well known fact that amino acids have inherent anti-inflammatory and anti-arthritic activity. Amino acids like *L*-tryptophan, *L*-histidine and *L*-glycine are carriers that have marked activity of their own.

3.3. Glucosamine conjugate: The rationale behind use of glucosamine is to mask acidic functional group temporarily. Glucosamine is an amino sugar which is physiologically used by the body to produce natural joint components like joint lubricants and shock absorbers.

Despite extensive efforts for separating therapeutic effect from GI toxicity, the search for an ideal prodrug with a superior therapeutic advantage for clinical use still remains unmet for the NSAIDs. Further research is needed to design and identify prodrugs, which would be appropriate for clinical use in terms of stability, metabolism, toxicity and side effects. Instead of synthesizing new compounds which is a time consuming and costly affair, the designing of derivatives of existing clinically used NSAIDs is definitely an interesting and promising area of research. Moreover, as the metabolic profile of the liberated parent drug (after cleavage of the derivative in the body) would be already known, it could be advantageous to design derivatives of parent NSAIDs.[2]

It has been shown that quaternary ammonium compounds accumulated preferentially in certain avascular cartilaginous tissues on intramuscular injection.[3] It has been postulated that these quaternary ammonium compounds are localized in the cartilage tissues, probably by virtue of ionic interactions with cartilaginous tissues.[3-4]

The localization of these quaternary ammonium compounds in the articular and epiphyseal cartilage and joint spaces might be of significance in relation to the distribution of drugs to inflamed joints and lesions of articular cartilage, and raised the possibility of enhancing the duration and intensity of anti-inflammatory effect in the inflamed cartilaginous tissues by chemically designing the drug derivatives by combining the antiarthritic activity and the joint localizing property.

From the literature it was concluded that cationic group i.e. pyridinium or ammonium ion is essential in such derivatives to show tropism towards cartilage[3-4], but ionic compounds specially cationic, are repelled due to positive charge present on the outside wall of the gastrointestinal membrane[5], as a result cations with a high kinetic energy or small size only are able to penetrate the ionic barrier. Another means of penetration is via ion-pair transport mechanism or as some complex with endogenous substance using active transport. Thus, at a given *p*H the rate of permeation of different molecules is in the following order-*unionized moleculs* > *anions* > *cations.* [6-8]

Due to the above given reasons, ionic derivatives may have low to poor rate of absorption through GIT membrane, which adversely contributes to their oral bioavailability. So there is no alternative to administer these derivatives other than parenteral route. As per literature all such derivatives have been administered by parenteral route only, but from the pharmaceutical point of view this is not a convenient and safe route.

2. Aims and Objectives

Oral delivery of NSAIDs for the treatment of arthritis is a widely accepted delivery system but it produces side effects during the treatment due to local GIT irritation and widespread distribution of the drug throughout the body. So, in order to avoid these side effects, delivery systems with the ability to deliver the drug to the required site is essential.

All the NSAIDs have one or more of the side effects such as GIT toxicity, renal adverse drug reactions (ADRs), blockade of platelet aggregation etc. Conventional NSAIDs have short biological half life needing frequent dosing to maintain the therapeutic efficacy for an extended period of time, which results in exhalted side effects. Due to these reasons, the development of new NSAIDs without these side effects has long been awaited but unfortunately this has not happened. Hence, the present work was undertaken with the aim of developing oral drug delivery systems of conventional NSAIDs to give site specific delivery for the treatment of arthritis, mainly RA and OA.

It was evident from the literature that cationic molecules possess affinity towards cartilage tissue which is anionic in nature. But such molecules show poor bioavailability on oral administration. Hence it was planned to convert the conventional NSAIDs in to neutral chemical delivery systems which would be easily absorbed from the GIT. Once they enter in to systemic circulation they should be metabolized/converted in to cationic species. Having a specific affinity for the anions these cations could be targeted to the negatively charged cartilaginous tissues. Once bound to the cartilaginous tissue of the joints, they should get hydrolyzed slowly into the original parent NSAIDs exhibiting their known therapeutic effects in the joints.

To achieve the above described aim it was planned to prepare dihydropyridine derivatives of the NSAIDs. The dihydropyridine derivatives would show neutral characteristics in the intestine and hence would be absorbed due to their non-ionic characteristics. The dihydropyridine derivatives would be oxidized in to quaternary derivatives by NAD-NADH co-enzyme system and would be concentrated in to inflamed joints due to ionic interactions as shown in **fig. 2.1.**

Adopting the above described approach, the problem of poor oral bioavailability could easily be circumvented and after metabolic conversion site specificity of the NSAIDs to the cartilaginous tissue could be achieved. In nutshell, the newly synthesized derivatives could achieve the designed aims due to the following reasons:

- The free carboxylic group of the NSAIDs would be masked into ester/amide functional group avoiding local GIT irritation.

- The neutral ester/amide derivative would be easily absorbed from the intestine.
- The dihydropyridine grouping present in the derivative would be oxidized to quaternary pyridinium ions thus generating the cation bound NSAIDs having high affinity towards the inflamed joints.
- Random distribution of conventional NSAIDs would be minimized by selective delivery of NSAIDs to the site of action.
- Site specific delivery of the NSAIDs to the affected joints would reduce the effective dose of the NSAIDs leading to further reduction in the toxic side effects.
- Chemical/enzymatic hydrolysis of the derivatives in the inflamed area would lead to sustained specific release of the parent NSAIDs.

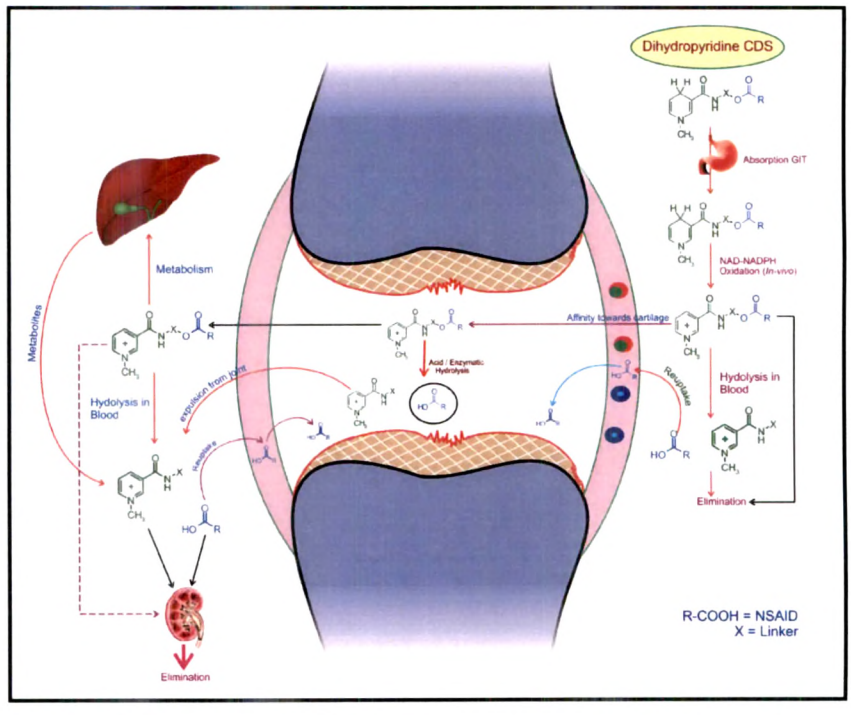


Fig. 2.1: Dihydropyridine Chemical delivery system

The following research activities were planned to be performed to achieve the laid down aims and objectives of the work:

I. To synthesize dihydropyridine derivatives of some common NSAIDs possessing ester or amide linkage.

II. To evaluate the synthesized derivative for their chemical/enzymatic susceptibility/stability.

III. To study the biodistribution of the quaternary derivatives using radiolabeling tags.

3. Results and Discussion

The work carried out towards achieving the proposed plan has been discussed under the following three main headings:

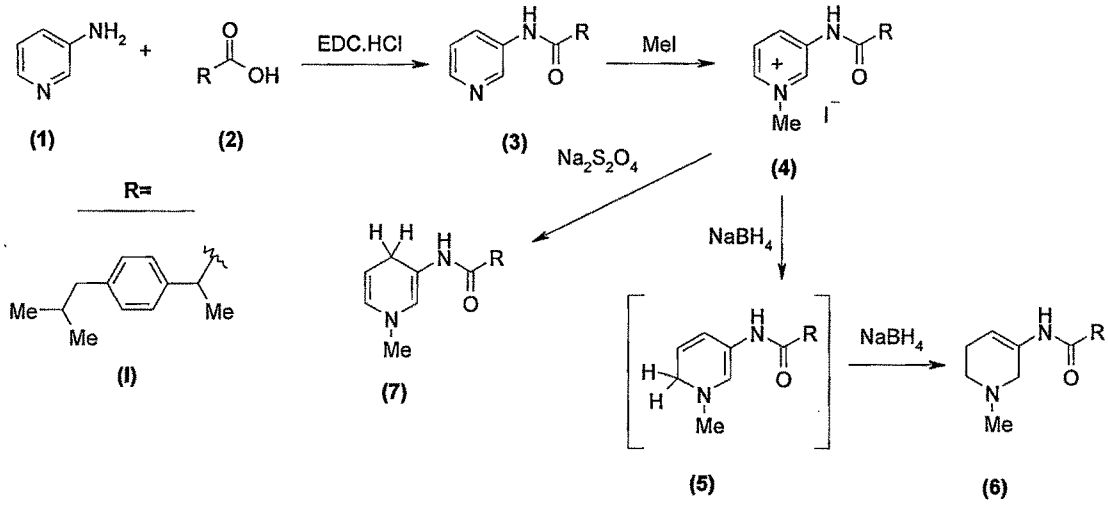
3.1. Chemical studies

3.2. Hydrolyses kinetics

3.3. Biodistribution and gamma imaging studies

3.1 Chemical studies

To synthesize the envisaged chemical delivery system (CDS), it was planned to couple the NSAIDs **(2)** with 3-aminopyridine **(1)** using EDC.HCl as the coupling agent to obtain the respective amide intermediate **(3)**. Quaternization of the pyridine nitrogen was planned by using methyl iodide. To obtain the desired dihydropyridine system **(7)** it was thought of using some mild reducing agent like sodium dithionite. To initiate the research work ibuprofen **(2I)** was used as the NSAID **(2)**. The planned **Scheme-1** for the synthesis of the desired CDS **(7)** went well up to the quaternization step to afford the ammonium derivative **(4)** but reduction of the quaternary ammonium derivative **(4)** to afford the desired CDS **(7)** could not be achieved using sodium dithionite.

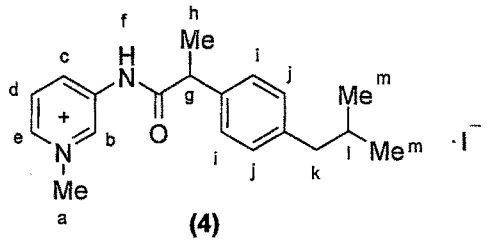


Scheme-1

The IR spectrum of compound **(3)** showed carbonyl stretching of amide group at 1693 cm^{-1} as sharp intense band. N-H stretching of amide was observed at 3282 cm^{-1}. The mass spectrum showed peak at 282.28 (M^+). This amide intermediate was quaternized using methyl iodide in acetonitrile or acetone as a solvent to obtain the quaternary ammonium derivative **(4)**. The IR spectrum **(Fig. 3.1. A)** of compound **(4)** showed

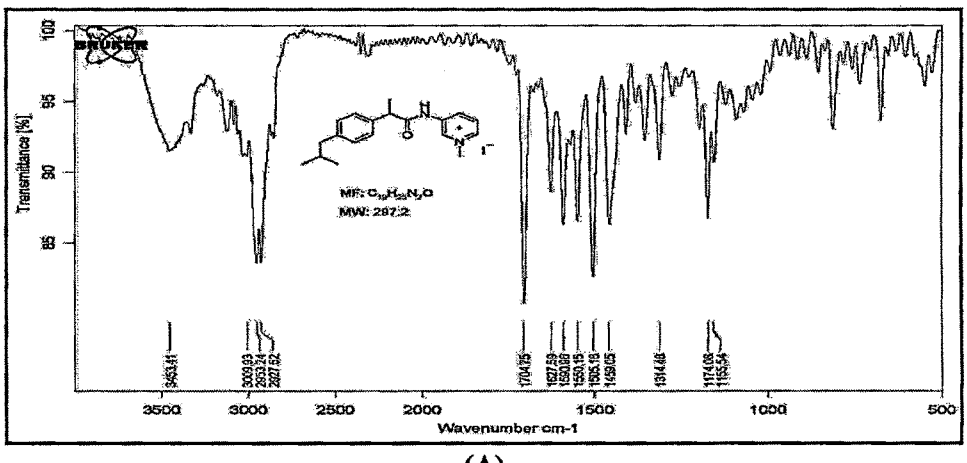
carbonyl stretching of amide group as sharp intense band at 1704 cm^{-1} and N-H stretching of amide was observed at 3453 cm^{-1}.

The PMR spectrum **(Fig. 3.1. B)** of the compound **(4)** showed singlet at δ 10.88 for single proton of pyridinium ring (-H_b), singlet at δ 9.75 due to amide-H_f, and a multiplet at δ 9.07-9.04 due to single proton of pyridinium ring (-H_e). Other protons of pyridinium ring appeared at δ 8.31-8.29 (-H_d) and δ 7.59-7.53 (-H_c) as multiplets.

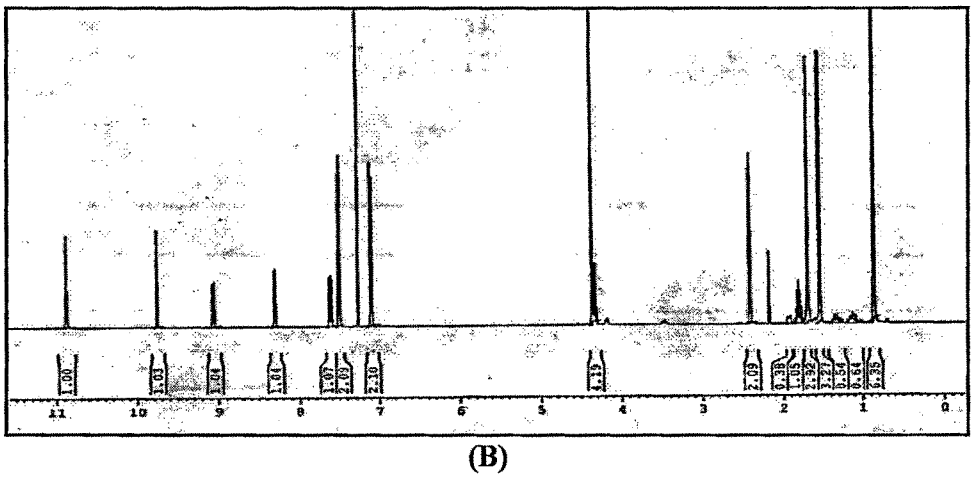


(4)

The protons of phenyl ring appeared at δ 7.51-7.08 as multiplet (Ar-H_{i-j}) and the methyl protons of quaternary nitrogen and -CH_g appeared at δ 4.36-4.30. The six methyl protons (-CH_{3m}) appeared at δ 0.87-0.85 as a doublet. The mass spectrum **(Fig. 3.1. C)** Showed peak at 297.1 (M^+).



(A)



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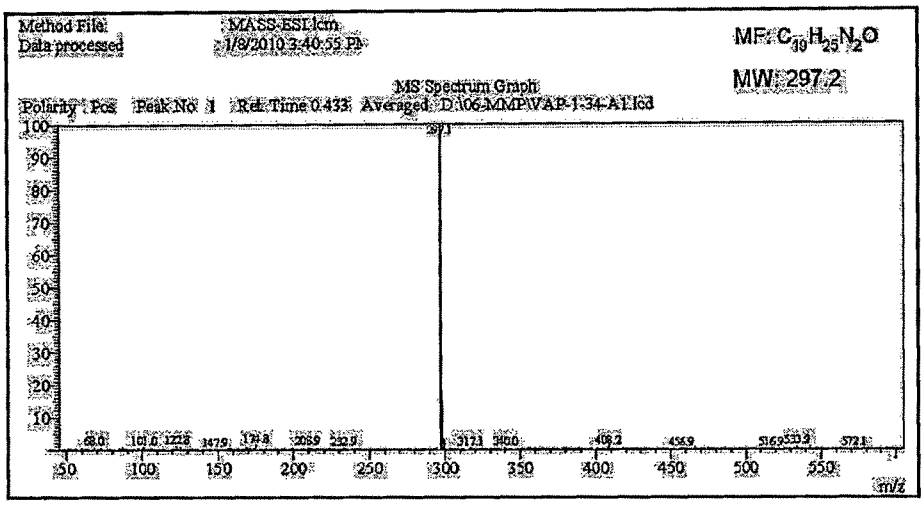
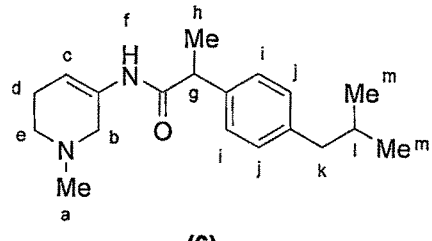


(C)

Fig. 3.1: Spectra of derivative **(4)**; IR spectrum **(A)**, ^{1}H-NMR **(B)** and Mass spectrum **(C)**

It was tried to reduce the quaternary derivative **(4)** using sodium dithionite under different experimental conditions but every time the starting material only was recovered back. After failing with dithionite it was thought of using a stronger reducing agent like sodium borohydride. After giving sodium borohydride treatment to the compound **(4)**, the product was isolated and characterized to be the 1,2,5,6-tetrahydro derivative **(6)** which was getting formed probably through the dihydro derivative **(5)** but even this dihydro derivative **(5)** could not be isolated.

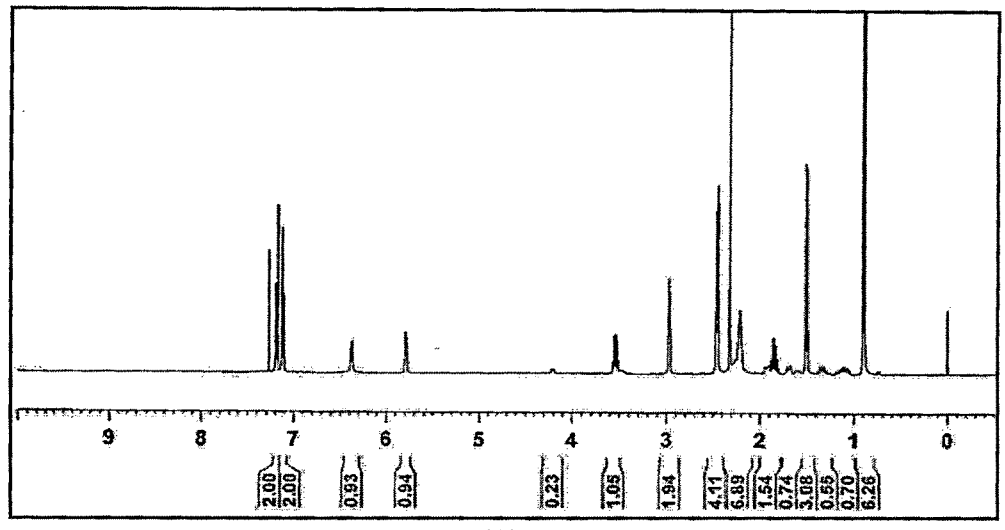
The tetrahydro derivative **(6)** showed strong peaks at 1665 cm^{-1} and 3505 cm^{-1} for carbonyl and N-*H* stretching vibrations in its IR spectrum.



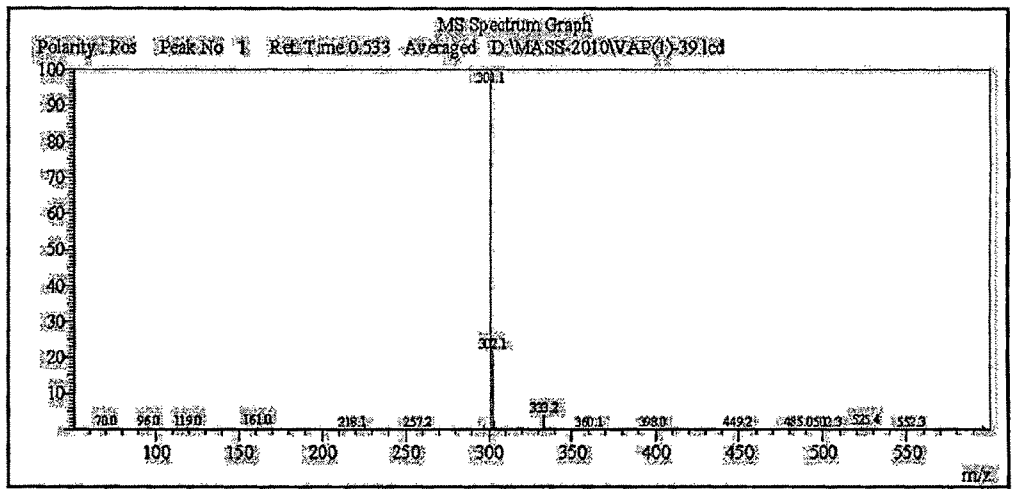
(6)

PMR spectrum **(Fig. 3.2. A)** of compound **(6)** showed the presence of aromatic protons at δ 7.19-7.11 (Ar-H_{i-j}), singlet at 6.23 due to N-H_f proton and the proton of cyclic alkene at 5.78 as multiplet (-H_c). Methyl protons on nitrogen appeared at δ 2.33 as a singlet (-CH_{3a}) and a doublet at δ 0.89-0.90 due to presence of dimethyl protons (-H_m). The mass spectrum **(Fig. 3.2. B)** showed peak at 300.1 (M^+). Its ^{13}C-NMR spectrum (**Fig. 3.2. C**) showed peaks at δ 172.7 for *C*=O carbon of amide groups, aromatic carbons at

140.96-110.23 and aliphatic carbons at 55.60-18.46. The H-H and C-H coupling was also confirmed using COSY and HETCOR spectra as shown in **Fig. 3.1. D-E**



(A)



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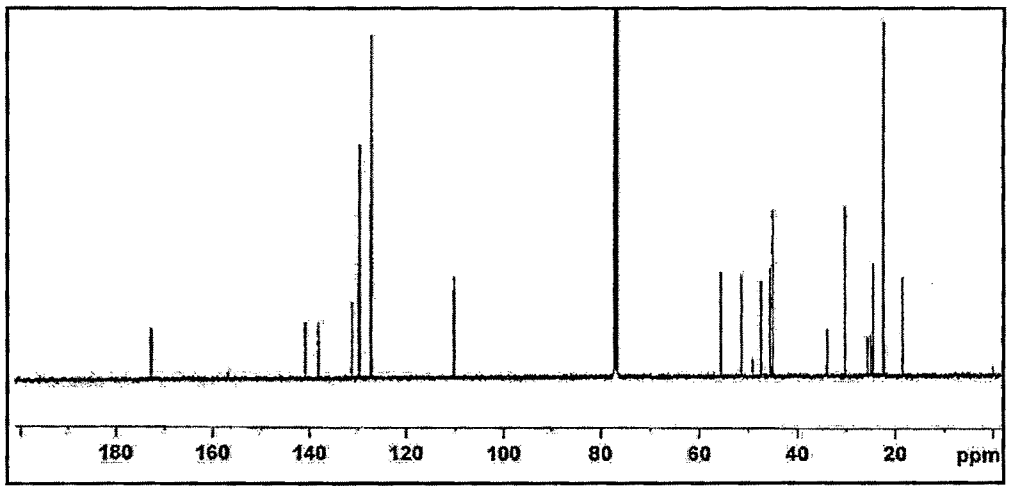

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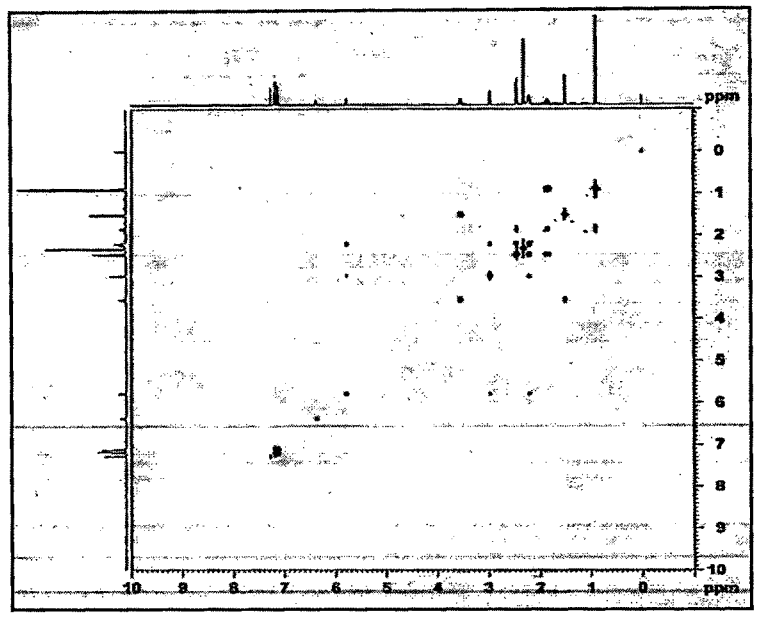
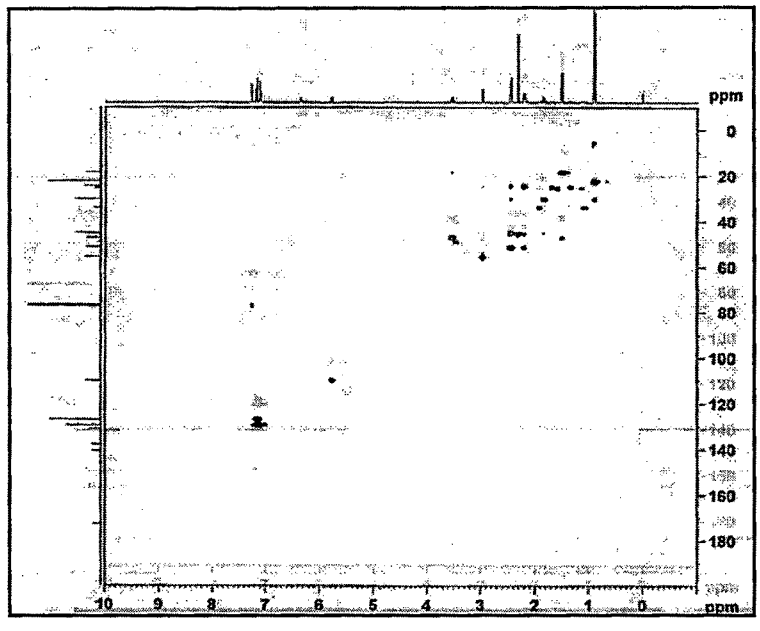


(D)



(E)

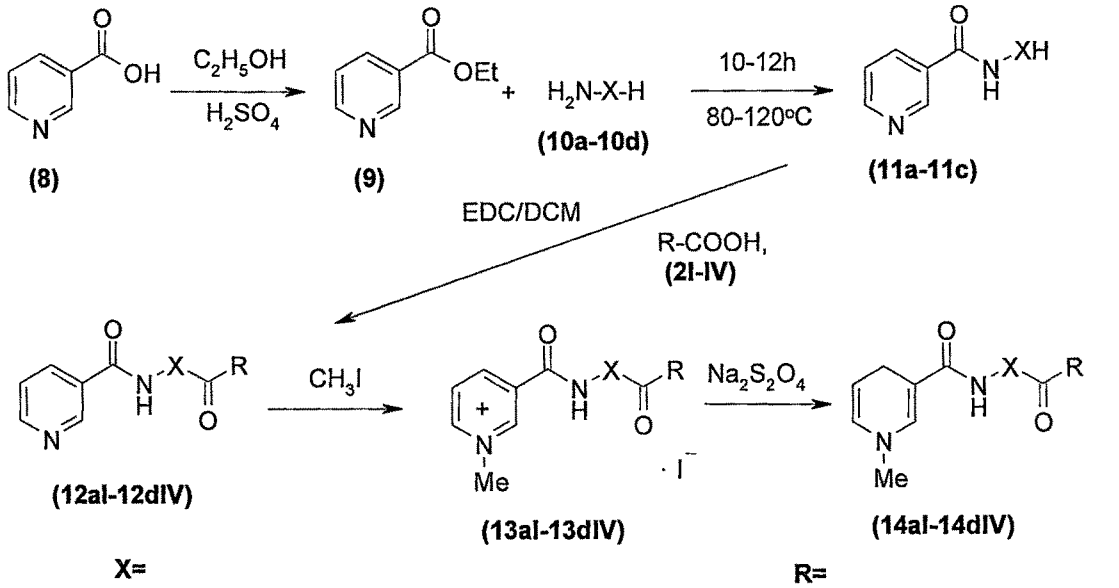
Fig. 3.2: Spectra of derivative **(6)**; ^{1}H-NMR **(A)**, ^{13}C-NMR **(B)**, Mass spectrum **(C)**, COSY **(D)** and HETCOR **(E)**.

From the literature it became known that reduction of substituted N-methyl pyridinium salts using sodium borohydride was easily achieved with regioselectivity. Pyridinium salts without electron withdrawing group **(4)** were reduced by sodium borohydride to give 1,2,5,6-tetrahydro derivatives, but not by sodium dithionite.[12] Reduction of these derivatives by sodium borohydride may take place in two ways, first direct reduction into 1,2,5,6-tetrahydro derivative **(6)** and the second one takes place

through two steps. First reduction offers 1,6-dihydopyridine derivative **(5)** followed by its further reduction in to 1,2,5,6-tetrahydro derivative **(6)**

Literature also revealed that substituents present on N-methyl pyridinium ring favor the regioselective reduction mediated by sodium borohydride as well as sodium dithionite. Electron withdrawing groups at position-3 on pyridine ring are necessary to give 1,4-dihydropyridine derivatives using dithionite as reducing agent while, derivatives without electron withdrawing groups are not reduced by dithionite at all but reduced by sodium borohydride to give tetrahydro derivatives[13-14]. So, it was planned to synthesize quaternary derivatives containing electron withdrawing group at position-3 which could be reduced by sodium dithionite to generate 1,4-dihydropyridine derivatives.

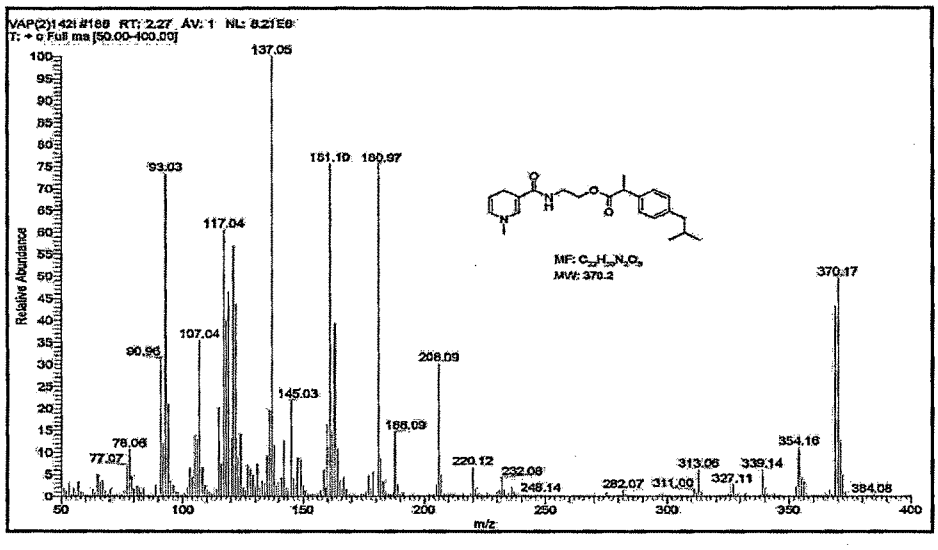
For the synthesis of the required derivatives containing electron withdrawing groups at position-3, nicotinic acid **(8)** was chosen as the starting material as shown in **Scheme-2.**



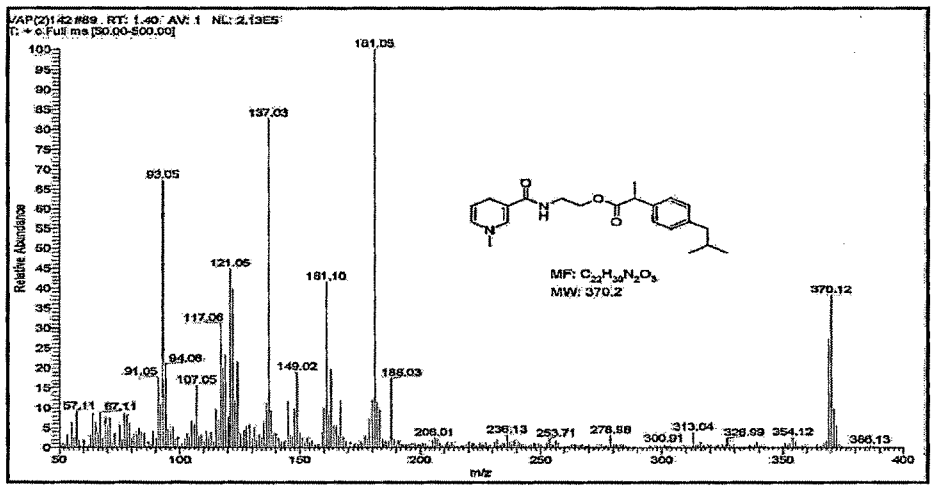
Scheme-2

Nicotinic acid was converted to ethyl nicotinate **(9)** and then treated with various aminoalcohols **(10a-10c)** or ethylenediamine **(10d)** at 100-120 ^{0}C to give the respective nicotinamide derivatives **(11a-11d)**. These intermediates were further coupled with various NSAIDs (**2I-2IV**) to offer the respective ester or amide derivatives **(12aI-12dIV).** These derivatives were then quaternized by methyl iodide in acetonitrile to generate the quaternary derivatives (**13aI-13dIV**). The detailed synthesis and characterization of these derivatives has been described under **Section-I**

Reduction of the quaternary derivatives **(13aI-13dIV)** was planned to be carried out using Sodium dithionite ($Na_2S_2O_4$) in an aqueous solution to afford 1,4-dihydropyridine derivatives **(14aI-14cIV).** From the NMR and mass data it became known that the synthesized dihydropyridine compounds were generally less stable than the corresponding quaternary derivatives.



(A)



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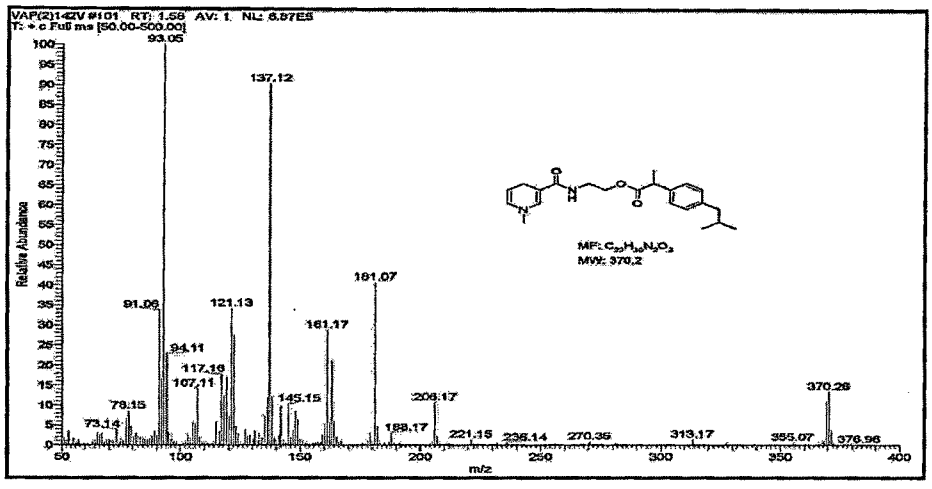

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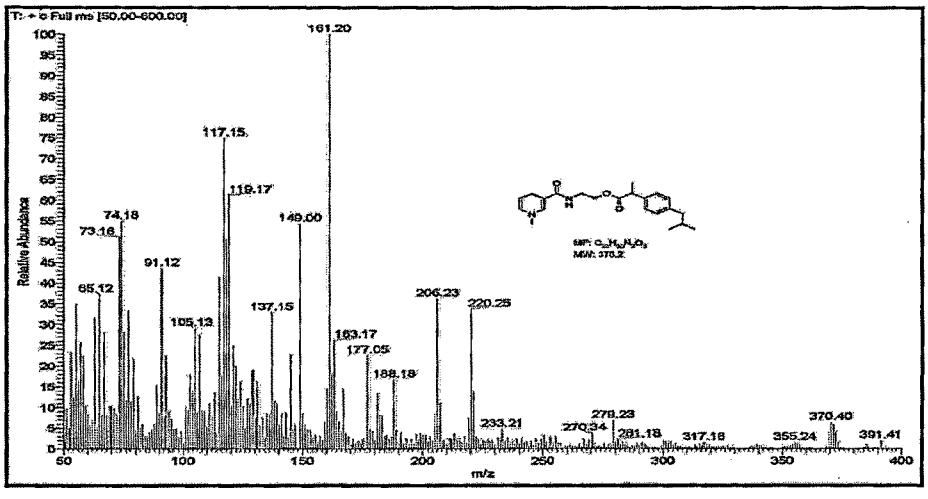


(D)

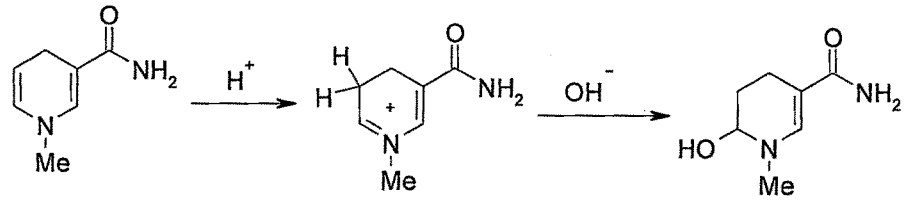
Fig. 3.3: A-D: Decrease in concentration of dihydropyridine derivative **(14aI)** with time.

The mass of the compounds showed gradual decrease in height of molecular ion peak and complete disappearance of the said peak occurred within 15-30 days. The decrease in peak height of mass spectrum with respect to time also confirmed instability of dihydropyridines as shown in **Fig. 3.3 A-D.** This instability of dihydropyridine derivatives was due to oxidation of the dihydropyridine ring system.[15]

Attempts to stabilize the dihydropyridine derivatives was made by salt formation approach using weak organic acids such as oxalic acid, succinic acid, as well as strong acids such as hydrochloric acid but without success.

It became evident from the literature that 1-alkyl 1,4-dihydropyridines are unstable and undergo facile oxidation (hydride loss) to the corresponding quaternary salts, but oxidation is not the only one process which is responsible for the instability of

dihydropyridines. It has been reported that 3-substituted-1,4-dihydropyridines such as **(15)** gets readily hydrated, especially in acidic media.[16-17] The mechanism of hydration involves a proton transfer from the acidic species to the C^5 position of the substrate, followed by a rapid attack by a hydroxy ion or other nucleophile at the C^6-position.[17-18]



(15) **(16)** **(17)**

Fig. 3.4: Mechanism of water addition reaction to 1,4-dihydropyridines

Oxidation of dihydropyridines is the dominant process under physiological conditions (*p*H 7.4). Susceptibility of the enamines toward water may seriously limit the possibility of developing CDS in aqueous formulations with pharmaceutically acceptable shelf-life. Water is readily and irreversibly added to the 5,6-double bond of 3-substituted-1-alkyl-1,4-dihydropyridines **(Fig.3.4)**, and the resulting 6-hydroxy-1,4,5,6-tetrahydropyridine derivatives are no longer subject to the enzymatic oxidation that could convert them into the desired quaternary forms.[19]

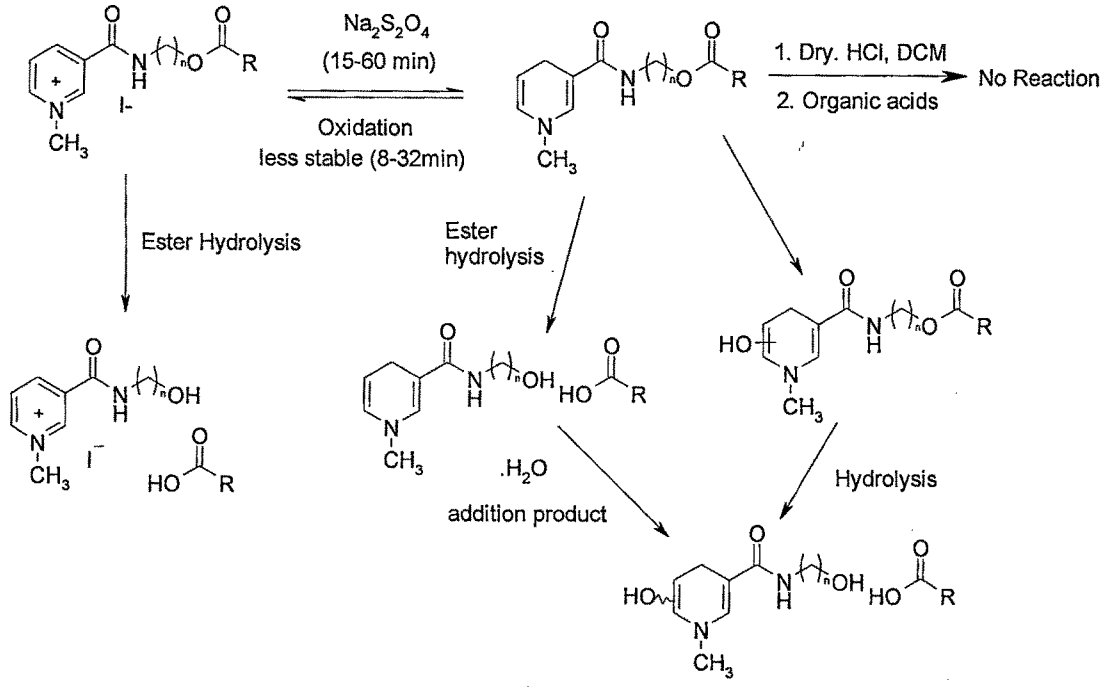


Fig. 3.5: Reduction of pyridinium salt and decomposition pattern of dihydropyridines

It was concluded that dihydropyridines followed much more complicated pattern than we imagined.[20] Dihydropyridines would show addition of water molecules to the 5,6-double bond, get oxidised to pyridinium salts, would exhibit hydrolysis of ester bond, and a combination of such processes. All these routes to liberate the parent drug are depicted in **Fig. 3.5.**

The possible protonation sites and mechanism of water addition in dihydropyridines is predicted by bodor *et al.*[21] as shown in **Fig. 3.6.** The addition of water to 1,4-dihydropyridines is chemically an enamine hydration type of reaction. It is known that electron donating (+*I*) substituents on the enamine nitrogen or carbon increase the reactivity of dihydropyridines towards hydration.[21]

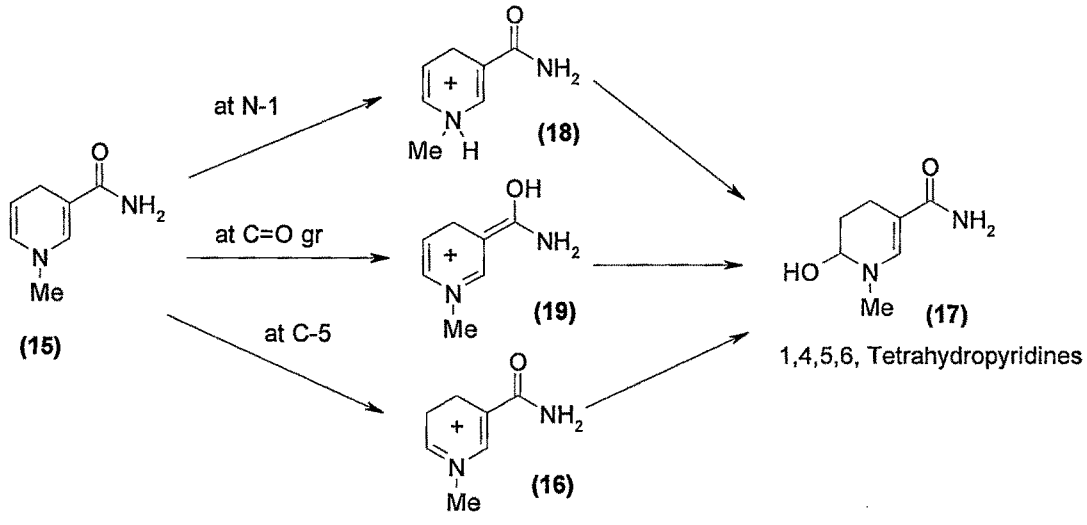
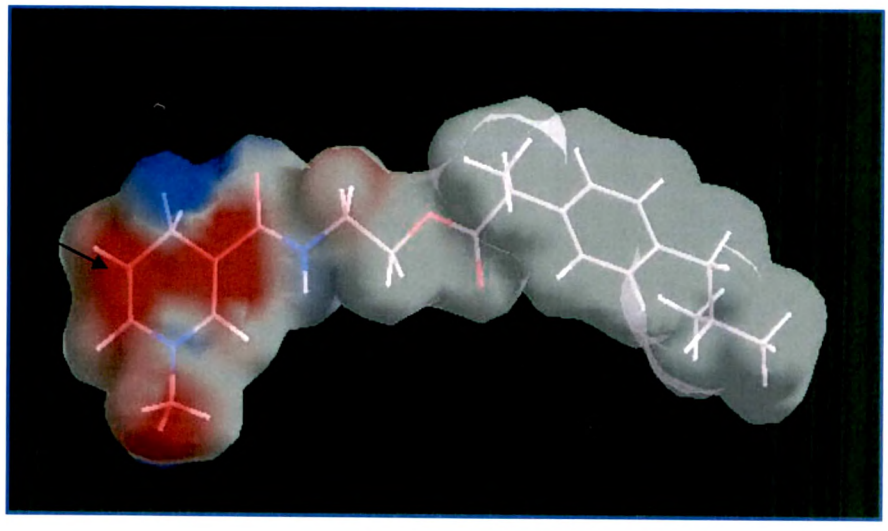


Fig. 3.6: Mechanisms of water addition in dihydropyridines

The possible protonation site was further confirmed by using AM1-based[22] semiempirical quantum chemical calculation. It show the C^5 position as the site with highest proton affinity in this moiety.[23] Furthermore, **Fig. 3.7** which represents the AM1-optimized structure of dihydropyridine derivative **(14aI)** covered with a soft surface, colored according to the electrostatic potential calculation based on AM1-charges. It shows a clear accumulation of electron density around the C^5-position, the site most susceptible to proton attack.[19]

Among the ibuprofen series, only one derivative **(14cI)** was found to be comparatively more stable. The IR spectrum of compound **(14cI)** showed carbonyl stretching of ester and amide groups as sharp intense bands at 1731 cm^{-1} and 1643 cm^{-1} respectively. N-H Stretching of amide was observed at 3301 cm^{-1}. Its mass spectrum **(Fig. 3.8.)** showed peak at 398.17 (M^+).

(A)



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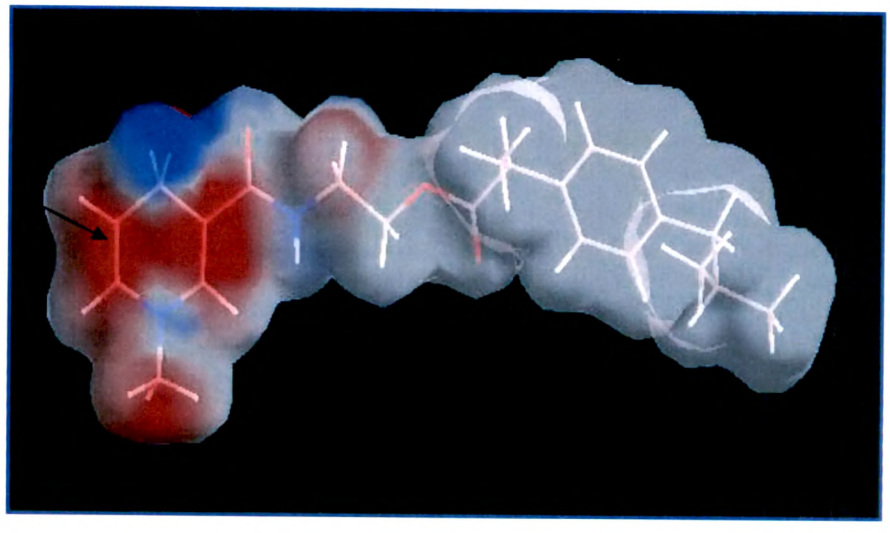

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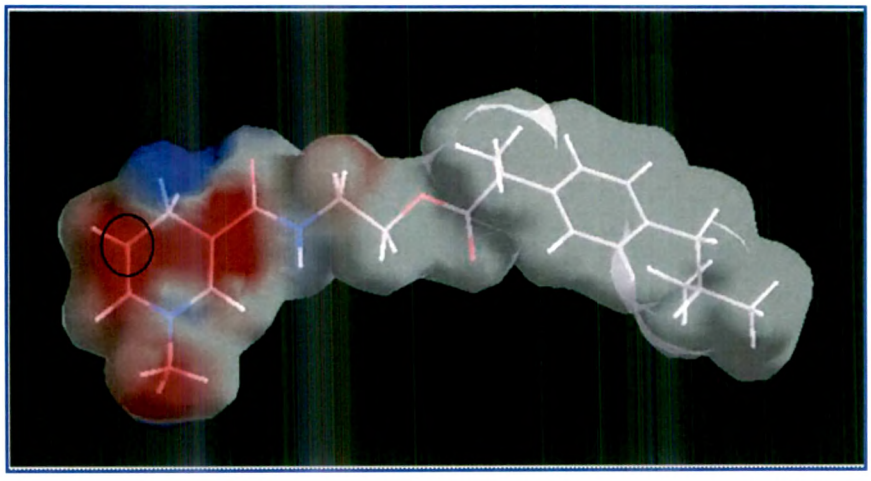


(D)

Fig. 3.7 A-D: AM1 optimized structure of dihydropyridine derivative **(14aI)** covered with a soft surface colored according to the electrostatic potential calculation based on AM1 charges. *(Region of increased electron density is found around C^5 even when compared to the basic N^1 atom)*

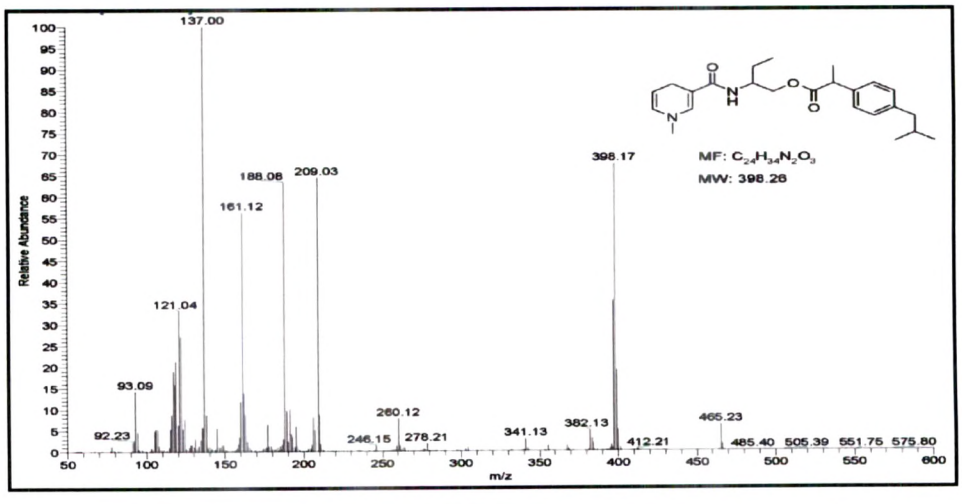
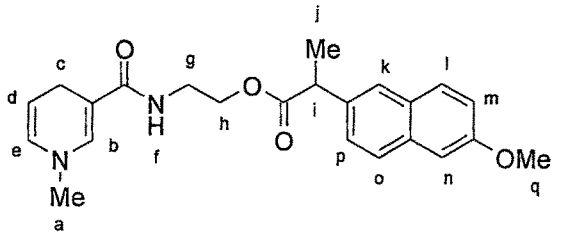


Fig. 3.8: Mass spectrum of **14cI**

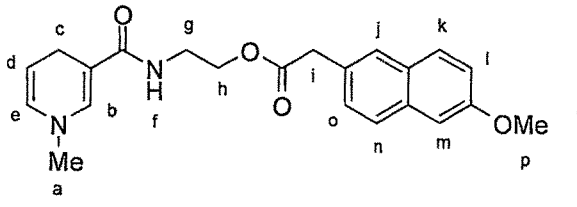
Other dihydropyridine derivatives **(14bII-14dIV)** obtained in **Scheme-2** were also found to be unstable and slowly got oxidized or degraded in the same way as shown in **Fig. 3.5**. Out of all these synthesized derivatives three derivatives **(14aII-14aIV)** were having relatively higher stability. These were further characterized and evaluated. The IR spectrum of compound **(14aII)** showed carbonyl stretching of ester and amide groups as sharp intense bands at 1728 cm^{-1} and 1638 cm^{-1} respectively. N-H Stretching of amide was observed at 3330 cm^{-1}.

The PMR spectrum of the compound **(14aII)** showed multiplet at δ 7.64-7.0 for naphthalene protons equivalent to six protons (Ar-H_{k-m}). Singlet at δ 5.22 appeared due to the dihydropyridine proton (-H_b). Another proton of dihydropyridine ring was observed at δ 4.1-4.0 as a multiplet (-H_d) and a peak at δ 3.84 was due to methyl protons present on nitrogen. Its mass spectrum showed peak at 394.31 (M^+).



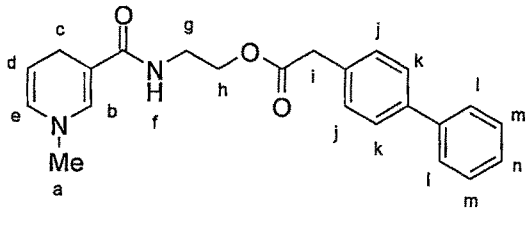
14aII

The IR spectrum of compound **(14aIII)** showed carbonyl stretching of ester and amide groups as sharp intense bands at 1731 cm^{-1} and 1684 cm^{-1} respectively. N-H Stretching of amide was observed at 3338 cm^{-1}.



14aIII

The PMR spectrum of the compound **(14aIII)** showed multiplet at δ 7.64-7.28 for naphthalene protons equivalent to six protons (Ar-H_{j-o}). Singlet at δ 6.83 appeared due to dihydropyridine proton (-H_b) and another proton of dihydropyridine ring was observed at δ 4.42-4.38 as a multiplet (-H_d). Peak at δ 3.67 was due to methyl protons present on nitrogen. Singlet at δ 2.65 appeared due to methylene protons (Ar-CH_2).



14aIV

The IR spectrum of compound **(14aIV)** showed carbonyl stretching of ester and amide groups as sharp intense bands at 1734 cm^{-1} and 1679 cm^{-1} respectively. N-H Stretching of amide was observed at 3223 cm^{-1}. The PMR spectrum of the compound **(14aIV)** showed multiplet at δ 7.52-7.19 for biphenyl protons equivalent to nine protons

(Ar-H_{j-n}). Singlet at δ 6.87 appeared due to dihydropyridine proton (-H_b) and another proton of dihydropyridine ring was observed at δ 5.56-5.54 as a multiplet (-H_d). Singlet at δ 2.65 appeared due to methylene protons (Ar-CH_2). Mass spectrum showed peak at 377.1 (M^+).

3.2 Hydrolyses kinetics study

All the synthesized CDS were evaluated *in vitro* for their stability at 37±1 °C in buffers of *p*H 2.0 and 7.4 which simulated the *p*H of the stomach and the blood. To get an idea about the enzymatic susceptibility of CDS towards serum esterase, in vitro hydrolyses studies were performed in pooled human serum (80/90 %) at 37±1 °C for all the CDS. HPLC methods were developed for the determination of half life of disappearance of the prodrugs over a definite period of time. The half life of disappearance of dihydropyridine CDS at various conditions are shown in **Table 3.1**.

Table 3.1: Half lives of disappearance of derivatives at various conditions

Derivative	Half life of disappearance of various CDS (min)		
	***p*H 2.0**	***p*H 7.4**	**Human serum**
14aII	12	18	54
14aIII	09	21	41
14aIV	11	14	46

From the above results it could be concluded that dihydropyridine CDS were quite unstable in phosphate buffer *p*H 2 as well as 7.4; the primary route of hydrolysis of these compound may be ester bond cleavage. All the synthesized CDS have undergone enzymatic hydrolysis in human serum to cleave ester bond present in CDS **(14aII-14aIV)**. From the table we can conclude that synthesized CDS were found to be comparatively more stable in human serum than in buffers of *p*H 2.0 and 7.4.

3.3 Biodistribution and gamma imaging studies

The radiolabeling of the compounds with reduced ^{99m}Tc was carried out as per the direct labeling method. The radiolabeling was optimized by taking three factors into account i.e. effect of *p*H on complex formation, incubation time and stannous chloride concentration. The *in vitro* stability of radiolabeled complexes was tested in human serum and in saline.

Swiss albino mice were used for the biodistribution studies of the ^{99m}Tc-labeled compounds (**14aII-14aIV**). Site specificity of CDS was studied in inflammatory condition. Animals were divided into two groups. **Group-A** (standard) was administered by oral route the parent NSAID **(2I)**. Another group **Group-B** (Test) was administered by oral route the synthesized dihydropyridine CDS, 3 h after induction of inflammation in the rat paw. A 200 µl of the ^{99m}Tc- labeled complex was administered through the oral route in each mouse. Blood was obtained by cardiac puncture at different time intervals, animals (group of three mice for each time interval) were sacrificed, different organs were dissected out, washed with normal saline, made free from adhering tissues, weighed, and the radioactivity measured in a shielded-well gamma scintillation counter. The percent radioactivity for each organ was calculated, and considering 7.3% of the total body weight as whole blood, calculations were performed for determining the radioactivity for whole blood **(Table 3.2 to 3.3).**

Table 3.2: Biodistribution of compound **(14aII)** in mice

Organ/ Tissue	**Percent injected dose/whole organ or tissue (±S.E.M.)**	
	1 h	**4 h**
Blood	2.62 ± 0.19	2.08 ± 0.25
Heart	0.63 ± 0.10	0.93 ± 0.21
Lung	0.42 ± 0.09	0.38 ± 0.10
Liver	4.23 ± 0.34	10.47 ± 0.46
Spleen	0.68 ± 0.24	0.73 ± 0.29
Kidney	0.31 ± 0.08	0.34 ± 0.09
Intestine	4.61 ± 0.55	10.87± 1.45
Stomach	24.50 ± 3.25	17.33 ± 3.25

From the results obtained it could be conclude that stomach is the target site for dihydropyridine derivatives and free ^{99m}Tc in the body. The dihydropyridine derivatives may get converted to their water addition product and hence accumulate in stomach or passed in to intestine. The results of the biodistribution studies suggest that major fraction of the drugs is present in stomach or intestine and this may be due to oxidation of dihydropyridine ring to quaternary derivatives and or water addition product which will not be absorbed in to systemic circulation. Further, high radioactivity in stomach may be due to instability of complexes of compounds (**14aII**) *in vivo* in stomach at pH 2.0. But the free NSAID **(2I)** gets absorbed from the stomach to a very high extent.

Table 3.3: Biodistribution of compound (**14aIII**) in mice

Organ/ Tissue	Percent injected dose / whole organ or tissue (±S.E.M.)	
	1 h	4 h
Blood	3.55 ± 0.21	2.37 ± 0.45
Heart	1.22 ± 0.15	1.17 ± 0.31
Lung	0.66 ± 0.11	0.48 ± 0.14
Liver	6.41 ± 0.36	16.98 ± 0.54
Spleen	0.71 ± 0.28	0.79 ± 0.22
Kidney	0.44 ± 0.12	0.54 ± 0.11
Intestine	5.23 ± 0.84	11.57± 2.45
Stomach	27.78 ± 2.89	12.10 ± 4.12

Table 3.4: Biodistribution of compound (**2I**) in mice

Organ/ Tissue	Percent injected dose / whole organ or tissue (±S.E.M.)	
	1 h	4 h
Blood	8.01 ± 0.18	2.34 ± 0.05
Heart	0.11 ± 0.02	0.08 ± 0.01
Lung	0.93 ± 0.12	0.54 ± 0.07
Liver	19.66 ± 1.21	15.98 ± 0.85
Spleen	2.55 ± 0.33	1.56 ± 0.13
Kidney	1.08 ± 0.14	1.09 ± 0.19
Intestine	0.82 ± 0.15	1.39 ± 0.12
Stomach	0.36 ± 0.003	0.15 ± 0.09

It could be concluded from the biodistribution studies that the designed CDS are not suitable for oral administration as these are not properly absorbed from the GIT, may be due to their fast conversion to various oxidation products which are not absorbed through oral route.

4. Experimental

The experimental work has been divided into three parts:

4.1. Chemical studies

4.2. Hydrolyses kinetics

4.3. Radiolabeling studies

4.1 Chemical studies

HPLC analysis was performed using Shimadzu prominence system (Kyoto, Japan) consisting of LC-20AT Pump, and SPD 20 A detector. The chromatographic column used was phenomenex C-18, 250 mm length X 4.6 mm diameter with 0.5 μ particle size and Column temperature was maintained at 25-28 °C. Separation of analytes was performed under isocratic conditions at a flow-rate of 0.5-1.0 ml/min. The mobile phase consisted of phosphate buffer (PB, 15mm)-acetonitrile. Chromatographic separations were performed on columns using silica gel 100–200 mesh and neutral alumina, activity grade I. All reagents used were of analytical reagent grade obtained from S. d. fine chemicals, Spectrochem, Qualigens and Sigma-Aldrich.

HPLC methods were developed for the determination of half life of disappearance of a prodrug over a definite period of time. The solutions and the buffers used in the study were prepared in triple distilled water. The buffers used were prepared according to the procedure as given in USP-2007. All the chemicals used were of analytical reagent grade. Anhydrous sodium sulphate was used as drying agent wherever required for the drying of organic solutions.

4.1.1. 2-(4-Isobutylphenyl)-*N*-(3-pyridinyl)propanamide (3)

Ibuprofen **(2I)** (2.41 g, 11.70 mM) and EDC (2.43 g, 12.77 mM) in dry dichloromethane (25ml) were stirred in an ice-bath at 0-5 °C. 3-Aminopyridine **(1)** (1 g, 10.64mM) dissolved in dry DCM (20 ml) was added drop-wise in to the above solution over a period of 10-15 min under stirring and the reaction mixture was stirred further for 6-8 h at room temperature. Glacial acetic acid (2-3 ml, 10 %) was added in to the reaction mixture and the stirring continued for further 30 min. The resulting reaction mixture was filtered through cotton and the filtrate washed twice with water, sodium carbonate solution (2 x 20 ml, 5%) and finally with water again (3 x 20 ml). The organic layer was separated and dried over sodium sulfate filtered and the solvent removed to get pure white colored oil. (2.4 g, 80 %).

Anal.:

TLC : R_f 0.71 (Chloroform: Methanol; 1:0.3)

UV (MeOH) : 221 nm

IR (Neat, cm^{-1}): 3282, 1693, 1167 and 802

MS (m/z) : 282.28 (M+1)

4.1.2. 3-[2-(4-Isobutylphenyl)propanamido]-*N*-methylpyridinium iodide (4)

2-(4-Isobutylphenyl)-*N*-(3-pyridinyl)propanamide **(3)** (1 g, 3.54 mM) was dissolved in to di-isopropyl ether (30 ml) and excess methyl iodide was added and the reaction mixture refluxed for 2 h. The solvent and excess reagent were removed to get a yellow residue which was crystallized using a mixture of water and methanol to get yellow crystals of the salt **(4)**, (1.2 g, 79.81 %, m.p. 166-168 ^{0}C)

Anal.:

TLC : R_f 0.61 (Chloroform: Methanol; 1:0.3)

UV (MeOH) : 220 nm

IR (Neat, cm^{-1}): 3453, 1704, 1627, 1174 and 750

PMR ($CDCl_3$): δ 10.88 (s, 1H, Ar-*H*), 9.75 (s, 1H, N*H*), 9.07-9.04 (d, 1H, Ar-*H*), 8.31-8.29 (d, 1H, Ar-*H*), 7.63-7.59 (t, 1H, Ar-*H*), 7.51-7.49 (d, 2H, Ar-CH_2), 7.10-7.08 (d, 2H, Ar-CH_2), 4.36-4.30 (q, 4H, -CH_3, CH), 2.41-2.39 (d, 2H, -CH_2), 1.83-1.77 (m, 1H -C*H*), 1.54-1.53 (d, 3H, -CH_3) and 0.87-0.85 (6H, d, (CH_3)$_2$)

MS (m/z) : 297.1 (M^+)

4.1.3. *N*-(1,2,5,6-Tetrahydro-1-methylpyridin-3-yl)-2-(4-isobutylphenyl)propanamide (6)

To a stirred ice cold solution of 3-[2-(4-isobutylphenyl)propanamido]-*N*-methylpyridinium iodide **(4)** (0.1 g, 0.23 mM) in demineralsed and degassed water (60 ml) under nitrogen purging was added potassium hydroxide (0.026 g, 0.47 mM) followed by sodium borohydride (0.009 g, 0.23 mM) . The mixture was stirred under nitrogen for 30 min at 0 ^{0}C maintaining the *p*H in alkaline range. Aqueous layer was extracted with degassed and cold ethyl acetate (3 x 20 ml) and the organic layer washed with cold demineralised water (3 x 20 ml) and dried over sodium sulphate. The solution was filtered through cotton and solvent removed under reduced pressure to give reduction product **(6)**

as white solid, which was further crystallized from ether to give a white solid. (0.046 g, 65.44 %, m.p. 114-116 ^{0}C)

Anal.:

TLC : R_f 0.61 (Chloroform: Methanol; 1:1)

UV (MeOH) : 220 nm

IR (Neat, cm^{-1}): 3505, 1665 and 835

PMR ($CDCl_3$): δ 7.19-7.17 (d, 2H, Ar-C*H*), 7.13-7.11 (d, 2H, Ar-C*H*), 6.23 (s, 1H, N*H*), 5.78 (s, 1H, -*CH*, cyclic), 3.56-3.51 (q, 1H, -*CH*), 2.98 (s, 2H, -CH_2, cyclic), 2.48-2.45 (t, 4H, (-CH_2)$_2$), 2.33 (s, 3H, -CH_3), 2.24-2.19 (d, 2H,-CH_2), 1.88-1.81 (m, 1H, -C*H*), 1.51-1.49 (d, 3H,-CH_3) and 0.90-0.89 (d, 6H, (CH_3)$_2$)

MS (m/z) : 301.1 (M^+)

4.1.4. 2-(1, 4-Dihydro-1-methylpyridine-3-carboxamido)ethyl 2-(4-isobutylphenyl) propanoate (14aI)

To a stirred ice cold solution of **13aI** (0.5 g, 1.0 mM) in demineralised and degassed water (60 ml) under nitrogen purging was added sodium bicarbonate (0.5 g, 6.0 mM) followed by sodium dithionite (0.88 g, 0.54 mM). The mixture was stirred under nitrogen for 20-30 min at 0-2 ^{0}C maintaining the *p*H at approximately 7 by addition of sodium bicarbonate. The aqueous layer was extracted with degassed and cold dichloromethane (3 x 20 ml) and the organic layer washed with cold demineralised water (3 x 20 ml) and dried over sodium sulphate. The solution was filtered through cotton and solvent removed under reduced pressure to give dihydropyridine derivative **(14aI)** as a yellowish semisolid. (0.25 g, 67.02 %)

Anal.:

TLC : R_f 0.78 (Chloroform: Methanol; 1:0.1)

UV (MeOH) : 221 nm

IR (Neat, cm^{-1}): 3316, 1730, 1645 and 1161

MS (m/z) : 370 (M^+)

4.1.5. 2-(1, 4-Dihydro-1-methylpyridine-3-carboxamido)ethyl 2-(6-methoxy-2-naphthyl)propanoate (14aII)

To a stirred solution of **13aII** (0.39 g, 0.75 mM) in demineralised and degassed water (150 ml) under nitrogen purging was added sodium bicarbonate (0.31 g, 3.75 mM) followed by sodium dithionite (0.39 g, 2.24 mM). The reaction was continued under

nitrogen for 20-30 min at RT. Then aqueous layer was extracted with degassed and cold dichloromethane or ether (3 x 20 ml) and the organic layer washed with cold demineralised water (3 x 20 ml) and dried over magnesium sulphate. The solution was filtered through Whatman filter and solvent removed under reduced pressure to give dihydropyridine derivative as a yellowish semisolid. The semisolid was further rinsed with ether and the solvent removed to get yellow oil which on standing got converted to yellow solid which was further purified by column chromatography to give dihydropyridine derivative **(14aII)** as a yellow semisolid. (0.20 g, 67.10 %).

Anal.:

TLC : R_f 0.78 (Chloroform: Methanol; 1:0.1)

UV (MeOH) : 221 nm

IR (Neat, cm^{-1}): 3316, 1728, 1638 and 1158

PMR ($CDCl_3$) : δ 7.64-7.0 (m, 6H, Ar-*H*), 5.22 (s, 1H, dihydropyridine-H_2), 4.1-4.0 (m, 2H, dihydropyridine-H_5, N*H*), 3.84-3.75 (m, 4H, N-CH_3), 3.43-3.36 (m, 3H, dihydropyridine-H_4), 2.94-2.73 (m, 3H, -CH_3), 2.0-1.97 (m, 2H, O-CH_2), 1.52-1.44 (m, 4H, O-CH_3, dihydropyridine-H_6) and 1.2-1.1 (q, 1H, -C*H*)

MS (m/z) : 394.31 (M^+)

4.1.6. 2-(1, 4-Dihydro-1-methylpyridine-3-carboxamido)ethyl 2-(6-methoxy-2-naphthyl)acetate (14aIII)

To a stirred solution of **13aIII** (0.50 g, 0.98 mM) in acetonitrile (5 ml) and demineralised and degassed water (150 ml) under nitrogen purging was added sodium bicarbonate (0.41 g, 4.90 mM) followed by sodium dithionite (0.51 g, 2.96 mM). The reaction was continued under nitrogen for 20-30 min at RT. Then aqueous layer was extracted with degassed and cold dichloromethane or ether (3 x 20 ml) and the organic layer washed with cold demineralised water (3 x 20 ml) and dried over magnesium sulphate. The solution was filtered through Whatman filter and solvent removed under reduced pressure to give dihydropyridine derivative **(14aIII)** as a yellowish semisolid. (0.25 g, 66.53 %)

Anal.:

TLC : R_f 0.78 (Chloroform: Methanol; 1:0.1)

UV (MeOH) : 221 nm

IR (Neat, cm^{-1}): 3338, 1731, 1684, 1168 and 852

PMR ($CDCl_3$): δ 7.64-7.28 (m, 6H, Ar-*H*), 6.83 (bs, 1H, dihydropyridine-H_2), 5.52-5.55 (d, 1H, dihydropyridine-H_6), 5.12 (bs, 1H, NH), 4.38-4.42 (m, 1H, dihydropyridine-H_5), 4.12-4.15 (m, 2H, dihydropyridine-H_5), 3.81-3.82 (m, 2H, N-CH_2), 3.83-3.84 (m, 2H, -CH_2), 3.66-3.67 (s, 3H, O-CH_3), 3.46-3.50 (m, 2H, O-CH_2), 2.81 (s, 3H, N-CH_3) and 2.64 (s, 2H, Ar-CH_2)

4.1.7. 2-(1, 4-Dihydro-1-methylpyridine-3-carboxamido)ethyl 4-biphenylacetate (14aIV)

To a stirred solution of **13aIV** (0.5 g, 0.99 mM) in acetonitrile (2-3 ml) and demineralsed and degassed water (150 ml) under nitrogen purging was added sodium bicarbonate (0.41, 4.90 mM) and sodium dithionite (0.51 g, 2.98 mM) once at a time. The reaction was continued under nitrogen for 30 min at RT. The aqueous layer was extracted with degassed and cold ethyl acetate (3 x 20 ml) and the organic layer washed with cold demineralised water (2 x 20 ml) and dried over magnesium sulphate. The solution was filtered through Whatman filter and solvent removed under reduced pressure to give dihydropyridine derivative **(14aIV)** as a yellowish solid (0.30 g, 80.15 %).

Anal.:

TLC : R_f 0.71 (Chloroform: Methanol; 1:0.1)

UV (MeOH) : 221 nm

IR (Neat, cm^{-1}): 3060, 1734, 1679, 835 and 753

PMR ($CDCl_3$): δ 7.52-7.19 (m, 9H, biphenyl-*H*), 6.87 (s, 1H, dihydropyridine-H_2), 5.56-5.54 (d, 1H, dihydropyridine-H_6), 5.21 (bs, 1H, N*H*), 4.50-4.47 (m, 1H, dihydropyridine-H_5), 4.33 (s, 2H, dihydropyridine-H_4), 4.17-4.15 (m, 3H, N-CH_3), 3.69-3.63 (t, 2H, O-CH_2), 3.53-3.51 (m, 2H, N-CH_2) and 2.85 (s, 2H, Ar-CH_2)

MS (m/z) : 377.1 (M^+)

4.2 Hydrolyses kinetics

All the synthesized CDS were evaluated *in vitro* for their stability at 37±1 °C in buffers of *p*H 2.0 and 7.4 which simulated the *p*H of the stomach and the blood. To get an idea about the enzymatic susceptibility of CDS towards serum esterases, *in vitro* hydrolyses studies were performed in pooled human serum (80/90 %) at 37±1 °C for all

the CDS. HPLC methods were developed for the determination of half life of disappearance of the prodrugs over a definite period of time.

Solutions and Buffers

1. Sodium hydroxide (0.2 M): Sodium hydroxide (0.8 g) was dissolved in distilled water and the volume made to 100 ml with distilled water.

2. Phosphate buffer (0.2 M): Potassium dihydrogen phosphate (2.722 g) was diossolved in water and made the volume up to 100 ml with distilled water.

3. Potassium chloride (0.2 M): Potassium chloride (1.491 g) was dissolved in distilled water and diluted to 100 ml.

4. Hydrochloric acid (0.2 N): Concentrated hydrochloric acid (1.7 ml) was diluted to 100 ml in a standard volumetric flask with distilled water.

5. Phosphate buffer *p*H 7.4: Potassium dihydrogen phosphate solution (0.2 M, 50 ml) was placed in a 200 ml volumetric flask and sodium hydroxide solution (0.2 M, 39.1 ml) added and the volume made up to 200 ml with distilled water.

6. Hydrochloric acid buffer *p*H 2.0: Potassium chloride solution (0.2 M, 50 ml) was placed in a 200 ml volumetric flask and hydrochloric acid (0.2 N, 13 ml) was added and the volume made with distilled water up to 200 ml.

Chromatographic conditions

Chromatography was performed under isocratic conditions, at a flow-rate of 0.75 ml/min. The mobile phase consisted of phosphate buffer (15 mM): acetonitrile (8:2). The solution was filtered through Whatman filter paper (0.2 μ) and degassed for 10 min in an ultrasonic bath. The column effluent was monitored at respective λmax. An aliquot of sample solution (20 μl) was injected onto the analytical column with a manual injection.

Calibration

An aliquot (20 μl) of each solution was then injected into the analytical column. All the measurements were performed in duplicate for each concentration The peak areas were measured and plotted against the respective concentration of the derivatives. Least square linear regression analysis was used to determine the slope, *y*-intercept and the correlation coefficients of the standard plots.

4.3 Radiolabeling studies

Swiss albino mice (a group of 3 animals for each time interval) were used for the biodistribution studies of the ^{99m}Tc-labeled compounds. The ^{99m}Tc-labeled complex (0.2 ml), prepared as described above, was administered through oral route to each mouse. At different time intervals (1 h and 4 h) the animals (group of three mice for each time interval) were anaesthetized and the blood obtained by cardiac puncture.

Blood was weighed and radioactivity measured in the gamma counter for each sample. The animals were sacrificed and tissues (heart, lung, liver, spleen, kidney, stomach and intestine) were dissected, washed with normal saline, made free from adhering tissues, weighed and their radioactivity measured. To correct for physical decay and to calculate radiopharmaceutical uptake in each organ as a fraction of the injected dose (% activity), aliquots (2.0 ul) of the complex solution, containing 2 % of the injected dose, were counted simultaneously at each time point. The percent activity for the whole organs and whole blood was determined for each time interval. The above described procedure was followed for studying the biodistribution of the complexes of compounds (**14aII-14aIII**).

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